REMARKS

Claims 134, 139-143, 145, 148-155 and 157-177 are pending. Favorable reconsideration is respectfully requested.

The rejection of Claims 153, 159, 162, 165, 169, 172 and 175 under 35 U.S.C. §103(a) over Longacre (1995) in view of Longacre et al (1994) is respectfully traversed. The cited references fail to suggest the claimed invention.

Longacre (1995) discloses the *Plasmodium cynomolgi* merozoite surface protein 1 C-terminal sequence and its homologies with other *Plasmodium* parasites. This reference also teaches that the C-terminal fragment of MSP-1 is a possible vaccine candidate. This reference fails to disclose that the C-terminal fragment of *P. cynomolgi* MSP-1 can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite and has the specific atomic coordinates in Annex I and the NMR fingerprints of Figures 12.0a to 12.0c.

This reference fails to disclose or even suggest a recombinant protein having a 19 kilodalton C-terminal MSP-1 fragment from Lys_{276} to Ser_{380}, as recited in Claim 153. Moreover, Longacre (1995) does not disclose a recombinant protein, which comprises further upstream a C-terminal end of p33 containing less than 50 amino acids (Claim 159), that the p33 is obtained from the cleavage of p42 (Claim 162) or an oligomer of the recombinant protein (Claim 169 and 172).

Longacre et al (1994) discloses producing recombinant MSP-1 proteins of 19-kDA and 42-kDA from *Plasmodium vivax*. More specifically, four recombinant constructs were produced with a 19-kDA and 42-kDA membrane-bound entity and a secreted 19-kDA and 42-kDA secreted entity lacking the membrane anchor in baculovirus. Longacre et al (1994) does not disclose that the recombinantly produced proteins can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite. This is evident from the last page of this reference,

where there is clearly an uncertainty with respect to whether the MSP1 C-terminal region plays any role in protective immunity.

Nor does this reference disclose a recombinant protein of *P. cynomolgi* with the specific atomic coordinates and NMR finger prints as currently claimed.

Moreover, this reference fails to disclose a recombinant protein having a C-terminal MSP-1 fragment from Lys_{276} to Ser_{380}, of SEQ ID NO: 11 of *P. cynomolgi*.

The combination of Longacre (1995) and Longacre et al (1994) does not render the presently claimed invention obvious since neither reference suggests or teaches that the recombinantly product proteins can inhibit parasitemia *in vivo* in a host infected with a *Plasmodium* parasite, a recombinant protein having a C-terminal MSP-1 fragment from Lys_{276} to Ser_{380}, which comprises further upstream a C-terminal end of p33 containing les than 50 amino acids (Claim 159), that the p33 is obtained from the cleavage of p42 (Claim 162) or an oligomer of the recombinant protein (Claims 169 and 172).

In fact, Longacre et al (1994) teach a C-terminal MSP-1 construct having 119 amino acids. In contrast, the presently claimed invention has 104 amino acids. There is simply no suggestion in either reference to modify the recombinant constructs either in length or by adding less than 50 amino acids of p33. Oligomers are not disclosed in either reference.

In view of the foregoing, Claims 153, 159, 162, 165, 169, 172 and 175 are not obvious over the cited references. Accordingly, withdrawal of this rejection is respectfully requested.

The rejection of Claims 134, 139 to141, 143, 145 and 148 to 150 under 35 U.S.C. §103(a) over Longacre (1995) in view of Longacre et al (1994) and further in view of Holder et al. (U.S. 5,720,959) is respectfully traversed. The cited references fail to suggest the claimed invention.

Longacre (1995) was discussed above. The same arguments apply to this rejection. More specifically, this reference fails to disclose that the C-terminal fragment of MSP-1 can inhibit parasitemia *in vivo* in a host infected with *Plasmodium* parasite and has the specific atomic coordinates in Annex I and the NMR fingerprints of Figures 12.0a to 12.0c (Claims 134 and 145), a recombinant protein, which comprises further upstream a C-terminal end of p33 containing less than 50 amino acids (Claims 139 and 176), that the p33 is obtained from the cleavage of p42 (Claim 142) or that the vaccinating composition is hydrosoluble (Claim 150).

Similarly, Longacre et al (1994) also fail to disclose that subject.

Holder et al (U.S. Patent 5,720,959) discloses polypeptides having the sequences shown in Figures 1 and 2, which are only individual EGF-like domains. These polypeptides do not contain any additional adjacent sequences in the MSP1 protein. Thus, the person skilled in the art would ascertain that only EGF-like sequences and nothing longer should be used in a vaccine composition. Thus, Holder et al teaches away from using any longer sequences.

The Holder et al patent fails to describe any atomic coordinates and NMR fingerprints as currently recited in Claim 134. These fingerprints are indicative of the precise folded structure of the 19 kilodalton C-terminal fragment used in the vaccinating composition and are indicative of the superior results achieved by the compositions of the present invention.

Moreover, as discussed in the previous response, Berghaus et al demonstrate that the EGF-like proteins described in Holder et al, which are expressed as fusion proteins in *E. coli* did not induce protection against a challenge of *Aotus nancymai* monkeys. Therefore no immune response in vivo was obtained.

There is simply no evidence in Holder et al. that alum can be used as an effective adjuvant in a malaria vaccine. Due to the unpredictability of the use of adjuvants with

selected antigens in the malaria vaccine art (see Annex I) the mere suggestion of the use of alum is inconsequential to this issue. Thus, only experimental proof would lead the skilled artisan to the fact that alum can be used as an adjuvant.

The combination of references fails to render the present invention obvious since none of the combined references specifically refer to the NMR fingerprints, which is indicative of the folded structure, which structure is important to achieve induction of an immune response. Moreover, evidence that alum works as an adjuvant with the antigens cited in the prior art is not proven and hence the mere suggestion would not lead the skilled artisan to conclude that this adjuvant has any worth; i.e., there is simply no expectation of success that alum would be an appropriate adjuvant.

In addition, none of the cited references describe or even suggest a vaccinating composition having upstream of the 19 kilodalton C-terminal fragment a polypeptide containing less than 50 amino acids (Claim 139) or less than 35 amino acids (Claim 141) of a C-terminal end of p33 of MSP-1 from a *Plasmodium* parasite. None of the cited references disclose or even suggest that the recombinant protein can be conjugated to a carrier (Claim 148).

Hence, even if a skilled artisan would combine Longacre (1995) with Lonagacre (1994) by substituting *P. vivax* with *P. cynolmolgi* and then use alum in a vaccinating composition there would be no expectation of success that an immune response could be achieved which inhibits parasitemia *in vivo* in a host infected with a *Plasmodium* parasite that is infectious in man. This is evidenced by Berghaus et al and the Holder patent, where an immune response to parasitemia was achieved in mice, but not in higher primates such as the *Aotus* monkey. *Aotus* monkey experiments are more indicative of how a vaccine composition would work in humans.

Thus, due to the lack of experimental evidence in the prior art and the unpredictability of adjuvants in this art, Applicants submit that the present invention is not obvious in view of the cited prior art. Accordingly, withdrawal of this ground of rejection is respectfully requested.

The rejection of the claims under 35 U.S.C. §103(a) over the combined teachings of Chappel and Holder, Miller et al, Longacre et al (1994) and Longacre (1995) is respectfully traversed. Those references fail to suggest the claimed invention.

Chappel and Holder describe that only the first growth factor like domain is the target of invasion inhibiting antibodies and that for all of the constructs tested against the antibodies, only the insect cell product produces superior results as compared to the bacterial produced products as demonstrated in Figure 2.

The baculovirus construct described in Chappel and Holder contains 271 amino acids of the Wellcome strain MSP1, including both EGF-like domains fused to the amino terminal 34 amino acids of MSP1. There is simply no incentive given in Holder and Chappell to alter this sequence. Moreover, this reference does not disclose using a leader sequence from *P. vivax*.

There is no indication in this reference that the first EGF-like domain can stimulate an immune response which can inhibit parasitemia in vivo in a host infected with a *Plasmodium* parasite. Indeed the last sentence of this reference would indicate to the skilled artisan that there has not been any testing with respect to the immunogenicity of the first EGF-like domain.

Furthermore, Chappel and Holder disclose that a change of glutamine to glutamate in the EGF-like domain results in the non-binding of the monoclonal antibodies that exists in *P. falciparum* invasion. In the presently claimed SEQ ID Nos. 1 and 4 glutamate are in the same position as taught by this reference. Hence to the person skilled in the art based on the

teachings of Chappel and Holder the presently claimed construct would have no growth inhibitory antibodies.

Miller is cited to orient sequences described in Chappell and Holder.

The comments regarding Longacre(1994) and Longacre (1995) apply to this rejection as well.

The combination of the present references fails to render the present invention obvious, since there would be no expectation of success that the constructs as currently claimed would in fact induce an immunogenic response. Indeed, Holder and Chappell discuss the notion that one change in the amino acid of the EGF-like region would in fact destroy or hinder the binding of monoclonal antibodies that inhibit *P. falciparum* invasion and hence would not be considered immunogenic. The one amino acid change in the EGF-like sequence is present in the presently claimed invention. Therefore, according to the cited references themselves, the claimed invention would not be immunogenic, which is contrary to the disclosure of the present invention.

Thus, lacking any expectation of success in the prior art references, this rejection cannot be maintained. Therefore, in view of the above, withdrawal of this rejection is respectfully requested.

The rejection of Claims 134, 139 to 143, 148, and 150 under 35 U.S.C. §103(a) over Chappel and Holder, Miller et al and Longacre (1995) in view of Longacre et al (1994) and further in view of Holder et al. is respectfully traversed. Those references fail to suggest the claimed invention.

Chappel et al, Miller et al and Longacre (1994) in view of Longacre et al (1995) were discussed in detail, and those comments apply to this rejection as well.

Although the Holder et al patent describes the possibility of using alum as an adjuvant in the vaccine composition, there is simply no demonstration that this adjuvant would work in

the patent. Indeed, it was known in the art at the time of filing the present application that malaria vaccines are very adjuvant dependent and the mere mention of an adjuvant for use without scientific demonstration that it can work has no real significance in this field. This is supported by the article "Vaccine Strategies" submitted herewith (marked Annex I).

Moreover, the combination of the references fails to render the present invention obvious since there would be no expectation of success that the claimed invention would in fact induce an immune response which inhibits parasitemia in vivo a host using alum as an adjuvant.

In addition, none of the cited references describe or even suggest a vaccinating composition having upstream of the 19 kilodalton C-terminal fragment a polypeptide containing less than 50 amino acids (Claims 139 and 176) or less than 35 amino acids (Claim 141) of a C-terminal end of p33 of MSP-1 from a *Plasmodium* parasite. None of the cited references disclose or even suggest that the recombinant protein can be conjugated to a carrier (Claim 148).

Applicants thus submit that the presently claimed invention is not obvious over the cited references. Accordingly, withdrawal of this rejection is therefore respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Annex I

Vaccine Strategies

Development of a vaccine towards malaria seems to be a definite possibility despite the fact that there are no vaccines in standard use against parasitic infections and the fact that malarial infection does not induce solid immune protection towards future exposure. During the late 1970's induction of solid immune protection had been achieved in humans though the use of irradiated sporozoites. However, the irradiated sporozoites must be delivered though irradiated, infected mosquitoes, and this was an impractical and too expensive method for widespread use. Even though natural infection of malaria does not produce complete immunity towards the parasite, long-term exposure to malaria has been shown to induce partially protective immune responses to malaria. In the partially immune there is fewer and less dense parasitemias, a reduction in malaria-related illness, significant protection from death, and antibody production towards erythrocytic stage parasites. An effective vaccine will probably need to incorporate multiple components that will induce an immune response towards the different stages of the malaria infection.

Life Cycle Stage	Immune Response
Sporozoite	- Abs that block hepatocyte invasion - Abs that kill the sporozoite via complement fixation or opsonization
Infected Hepatocyte	- CTL mediated lysis - CD4+ help for the activation and differentiation of CTL - Localized cytokine release by T cells or APCs - ADCC or C' mediated lysis
Asexual Erythrocytic	- Localized cytokine release that directly kills infected erythrocyte or intracellular pa - Abs that agglutinate the merozoites before schizont rupture - Abs that block merozoite invasion of RBCs - Abs that kill iRBC via opsonization or phagocytotic mechanisms - Abs engulfed with the merozoite at time of invasion which kill intraerythrocytic pa - Abs which agglutinate iRBCs and prevent cytoadherence by blocking receptor-liga interactions (CD-36 is such a receptor) - Abs which neutralize harmful soluble parasite toxins
Sexual erythrocytic	- Cytokines which kill gametocytes within the iRBC - Abs that kill gametocytes within iRBC via C' - Abs that interfere with fertilization

- Abs that inhibit transformation of the zygote into the ookinete

- Abs that block the egress of the ookinete from the mosquito midgut

(Woolan and Hoffman)

Pre-Erythrocytic Phase

One strategy towards the pre-erythrocytic stage is to target the parasite during the short span of time that the sporozoites are in the bloodstream. This sporozoite vaccine must induce the production of protective antibodies that will block and neutralize the sporozoites from invading liver cells. The other strategy is to target the sporozoites once they are inside the liver cells through the induction of CTLs that will destroy sporozoite infected liver cells.

Erythrocytic Phase

Another approach is to induce blocking antibody towards the circulating merozoites, preventing them from infecting red blood cells. Once inside the erythrocytes, CTL cannot be generated against them since red blood cells do not express MHC molecules on its surface. However, some malaria antigens are expressed on the surface of the infected RBCs toward which antibodies can be directed against and be used for opsonization and complement. Also, it may be helpful to induce antibodies that block the infected erythrocytes from adhering to the lining of blood vessels. It is during the erythrocytic stage that illness associated with malaria occurs. There are strategies, called 'anti-disease' vaccines, towards the toxic products produced during this phase.

Transmission Phase

There are also attempts to produce a 'transmission-blocking' vaccine. This approach targets the sexual stage gametocytes of malaria. The goal is to prevent the gametocytes from producing more sporozoites within the gut of the mosquito vector, thus blocking the transmission of malaria. This vaccine does not prevent illness in an infected host, but it may be important to reduce the spread of malaria.

Adjuvants

Creating a malaria vaccine does not only involve discovering the optimal antigens; it is also important to enhance the immune response towards those antigens through adjuvants, especially since adjuvants are usually required with non-living vaccines. Studies have shown that one of the main determinants of protection against malaria may be the adjuvant vehicle. The adjuvants may be essential in influencing the specificity and isotype of the desired antibodies. One study investigated the effectiveness of the CS protein conjugated to BSA in differing ratios. Even though antibody titers were comparable among different ratios,

that did not correspond with induced protection. It seemed that certain peptide-carrier ratios elicited antibodies with greater avidity to the desired antigen.

Freund's complete adjuvant has been relatively successful as an adjuvant for malaria vaccines, but this adjuvant is too toxic for human use. Other options for adjuvants may be mycobacterial cell wall skeleton, monophosphoryl lipid A, squalene, and liposomes. There has been much recent clinical success with SBAS2.1/SBAS2, which are coupled with MSA-1 in vaccine form. These adjuvants have a greater potency than Freund's adjuvant.

Cytokine injection in conjunction with vaccine administration has proven limited success.

[redacted]

VACCINE METHODS

SPf66 - the first recognized malaria vaccine. SPf66 was developed in Colombia by Manual Patarroyo in 1987 by purifying three merozoite-derived proteins and joining them with sequences derived from the repeat domain of the CS protein of *P. falciparum*. Phase I trials demonstrated a 75% rate of efficacy and showed that the vaccine was immunogenic and well tolerated. Phase IIb and III trials demonstrated an efficacy rate that ranged between 38.8-60.2%. The first trial in Africa was conducted in Tanzania in 1993, where intense malaria transmission occurs. The estimated vaccine efficacy rate was 31% after a one-year follow-up period. SPf66 was also confirmed to be safe and immunogenic. A later trial in the Gambia did not show any protective effect elicited by the SPf66 vaccine; however, factors such as the short three and a half months follow-up period were given as reasons for this outcome.

In recent studies, it has been determined that SPf66 has very low immunogenicity and induces only a temporary humoral immune response (6 months on average). The antibody subtype implicated is IgG. Among African children, the antibody titer following exposure to SPff66 rises with age, however this finding is tentative.

There is much controversy surrounding the SPf66 vaccine. Many have criticized the manner in which the trials have been conducted and the fact that it is not understood how SPf66 mediates protection. SPf66 will most likely not be the answer to the malaria burden, but it has given several research teams valuable experience in conducting malaria vaccine trials that can be helpful during the next generation of trials.

CSP - a vaccine based on the circumsporozoite protein (CSP) has been developed and field tested in humans in a malaria-endemic region of Kenya (reference). The vaccine incorporated the recombinant (Asn-Ala-Asn- Pro15 Asn-Val-Asp-Pro)2-Leu-Arg (R32LR) covalently linked to purified Pseudomonas aeruginosa toxin A 9. The intended outcome was an elevated T-lymphocyte response, however this was not observed in the study group. There was no observed reduction in incidence of disease in the study group as compared to the control group (which received hepatitis B vaccine). CSP vaccine recipients had an 82%